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BURYING AND MOLTING OF PINK SHRIMP, *PENAEUS DUORARUM* (CRUSTACEA: PENAEIDAE), UNDER SELECTED PHOTOPERIODS OF WHITE LIGHT AND UV-LIGHT

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Photoperiod is known to affect the behavior and physiology of arthropods. Photoperiodism has been more thoroughly investigated in insects than in crustaceans, and it was not until 1946 that Panouse found that photoperiod may affect certain crustacean physiological processes. Later the effects of light on the molt cycle were examined by Bliss (1954a, b) and Stephens (1955), their work clearly demonstrating a relationship among photoperiod, hormonal regulation, and the molting process. Although a large number of papers discussing the effects of light on crustacean behavior and physiology have been published (Black, 1963; Buikema, 1968; Bulnheim, 1966; Lowe, 1961; Rao and Nagabhushanam, 1967), the effects of photoperiod on the molt cycle have received little attention, as noted by Aiken (1969).

The onset of molt in crustaceans is controlled by X-organ secretions of the molt-inhibiting hormone (MIH). Because the X-organ is located in the eyestalk (Passano, 1960), it may be light sensitive. Thus, if the MIH can be controlled by photoperiod, level of light energy, specific wavelength, or a combination of these, ecdysis may be induced. Results from Aiken's (1969) work on the freshwater crayfish *Orconectes virilis*, demonstrate that X-organ secretions are, in fact, controlled by various photoperiodisms.

During the initial studies of testing various pelletized foods for the shrimp, *Penaeus duorarum*, bacterial buildup became an increasingly important problem

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with time. An effort to reduce the bacterial population was made by irradiating different aquaria with selected photoperiods of UV-light. Results indicated that shrimp irradiated daily with zero, 12, and 24 hr of UV-light exhibited an increasingly higher molt rate, respectively. These preliminary data warranted further investigation, and a more thorough study was undertaken to describe the effects of different photoperiods of white light and UV-light on the burying, growth, and molting of *Penaeus duorarum* in controlled conditions. This paper presents and discusses the burying and molting data.

The diurnal burying behavior of *P. duorarum* has been reported by Eldred (1958), Eldred, Ingle, Woodburn, Hutton, and Jones (1961), Fuss (1964), and Ogren (1966), Idyll (1950), Viosca (1957), and Williams (1958). Wickham (1967) studied the persistence of the burying behavior under experimental conditions, and Hughes (1968) sought the factors that were responsible for the shrimp's emergence from the substrate. To date, however, their observations and conclusions have not been reinforced with long term experiments and sound statistical analysis.

Materials and Methods

Shrimp used in the experiment were caught in shallow grass beds on August 21, 1969, midway between St. Teresa and Turkey Point, Franklin County, Florida, USA. The shrimp were placed in a seawater holding tank and selected for size and species. Pink shrimp, *Penaeus duorarum*, between 55 and 60 mm total length (*i.e.*, tip of rostrum to tip of telson) were used for the experiment because this size range allowed measurable growth rates over a period of several weeks and was also large enough to provide readily observed exuviae. Ten shrimp of similar size were placed in each tank. The shrimp were measured (total length) individually, and weighed in groups of ten, before being placed in aquaria and again at weekly intervals to determine growth rate.

Five groups of 38-liter aquaria (two/group) were exposed to different daily photoperiods: Group I received 0-hr light (00:24LD); Group II, 12-hr white light (12:12LD-W); Group III, 12-hr UV-light (12:12LD-UV); Group IV, 24-hr white light (24:00LD-W); Group V, 24-hr UV-light (24:00LD-UV). Aquaria of Group I (00:24LD) were placed in a black box (64×64×94 cm) to exclude all light (except during the monitoring periods), while the other groups were partitioned from each other by black cloth to prevent interference among the different photoperiods. Photoperiod refers to the light (L) and dark (D) cycle; photophase denotes the light phase, scotophase denotes the dark phase (Aiken, 1969). Photoperiods were controlled by an electric timer, and the photophase for Groups II (12:12LD-W) and III (12:12LD-UV) began at 1200 and ended at 2400 while the scotophase ran from 0000 to 1200. Ken Rad's fluorescent F 40 "Cool White Lamp" and General Electric's F-40-T-12-BLB "Black Light" lamp were used as a source of white light and UV-light, respectively. Spectral distributions of the lamps were obtained from the respective manufacturers. The spectral distribution of the white light was 400 to 700 nm; wavelengths of 520 to 660 nm contributed the most energy. The range of wavelengths for the UV-light was 300 to 400 nm. One lamp was placed approximately 30 cm above the water's

surface and centered perpendicular to the long axes of the aquaria. The aquaria of each group were placed juxtaposed with their long axes parallel.

Feeding and observing the shrimp took place twice daily, once between 1300 and 1500 and again between 0100 and 0300. Initially, at each feeding, shrimp were fed chopped frozen squid amounting to 5% of the shrimp's total weight. After two weeks, however, shrimp showed poor growth so the amount of food was doubled and remained at this level for the duration of the experiment. Just prior to feeding, exuviae were collected, counted, and recorded. Presence of any dead shrimp was noted, and any excess food removed to prevent contamination.

Because the exact stage of the molt cycle was at first unknown, a minimum of three exuviae per shrimp were examined to show the effects (if any) of the different photoperiods. Observations from preliminary studies revealed that approximately eight weeks are needed to obtain the desired number of exuviae for 55–60 mm shrimp (total length); hence, the experiments were continued for a duration of 56 days.

To ensure isotropic properties among the tanks, the water of the groups with an equal duration of photophase, *i.e.*, Groups II, III, and Groups IV, V, was interchanged. The water from the last tank in Group III or V was continuously pumped to the first tank in Group II or IV, respectively. Water circulated through the other tanks back to its original location *via* siphons, at flow rates approximating 75 liters/hr. Water was passed through under-gravel filters, and Silent Giant air pumps were used to supply aeration to the aquaria.

A two-cell flashlight (C-cell) with a red filter (> 550 nm) was used to observe the shrimp in Group I, and Groups II and III during the scotophase. Observations lasted less than 5 minutes a day for Group I and less than 2.5 minutes a day for Group II and Group III. Eldred (1958) found that red light caused least disturbance among active shrimp in the dark, and Fernandez (1965) found the maximum absorption of the eye pigments of *P. duorarum* to be at 516 nm. Growth rates were determined each week by measuring individual shrimp to the nearest mm (total length) and by weighing the shrimp in groups to the nearest hundredth of a gram. A triple-beam balance was used to obtain wet weights of the shrimp by placing them in a tared beaker of sea water and taking a weighted difference. In addition to the red flashlight, the shrimp in Group I were exposed to a maximum of 15 minutes of red light ($\lambda = 550$–750 nm) from an incandescent lamp once a week while they were being measured and weighed. The energy levels of the light used were measured by a YSI-Kettering Radiometer Model 65 with a YSI 6551 probe. The measured light energies at 30 cm were 2600, 100, 20000, and 16000 ergs $cm^{-2}sec^{-1}$ for the white light, UV-light, red filter and flashlight, and red incandescent light, respectively. Per cent transmission of light through 10 cm of aquaria water at the experiment's end was measured to be the following average values: white light (500–700 nm), 97; UV-light (300–400 nm), 85; red filter and flashlight, (> 550 nm), 98; and red incandescent light (550–750 nm), 98.

A Beckman-DK-2A ratio recording spectrophotometer was used to measure the transmission of light through the red filter and the aquarium water, and a Beckman DB-G grating spectrophotometer was used to obtain the transmission of light through a piece of the red incandescent lamp.

Temperature and salinity were maintained at 25° C and 25‰, respectively, because these conditions were found to be optimal for growth and survival of the brown shrimp, *P. aztecus* (Zein-Eldin and Aldrich, 1965), which is found in temperature and salinity ranges similar to those of *P. duorarum*. About 4 cm of sand-gravel mixture were used for a substrate because *P. duorarum* preferred this to other bottom types (Hildebrand, 1954; 1955; Williams, 1958).

Burying, growth, molt, and mortality data for the individual aquaria of each group were summarized into four two-week periods and an analysis of variance employing a split-plot arrangement in a completely randomized design was computed (Steel and Torrie, 1960). The two-week period burying, molt, and mortality data consisted of the cumulative number of shrimp above the substrate, number of exuviae, or mortalities divided by the cumulative number of shrimp in the tank at each observation, respectively. The effects of the treatments (*i.e.*, photoperiod), the periods (two-week interval), and the treatment × period interactions on average weight increases were evaluated. In a similar manner, the effects of the treatments, periods, times, and the treatment × period, the treatment × time, the period × time, and treatment × period × time interactions were also evaluated for the average burying, molt, and mortality rates. If a significant difference was found, then orthogonal comparisons (Snedecor and Cochran, 1967) were made to explain more specifically the difference among the treatments, periods, times, etc.

Throughout the text, references are made to shrimp being more active during the entire "photophase" or "scotophase," but observations lasted only a small fraction of these times, and in reality, cannot be extended to the entire time intervals. It is believed, however, that the data are indicative of the influence of the photoperiods on shrimp behavior in aquaria or culture conditions, but care should be exercised in interpreting the results and conclusions to wild populations of shrimp subjected to many additional and interacting variables. For convenience, the photoperiod is frequently given in parenthesis behind a particular group. The terms *treatment* and *group* are used synonymously.

Environmental factors

Initially it was not known whether the wavelength, the amount of energy, the photophase, or a combination of these factors affected the burying behavior, growth, and molt rate of shrimp. Because pink shrimp usually bury themselves during the photophase (Fuss, 1964), it is difficult to estimate how much energy, if any, they are receiving. The shrimp in groups with continuous light were not always buried and therefore were exposed to some light. Sometimes the shrimp buried themselves completely, while at other times their antennal scales, eyes, and rostrum were exposed. Therefore, the energy received by a shrimp is dependent upon its burying behavior.

Ideally the intensity of the lights should have been such that the groups with an equal photophase also received equal amounts of energy, so any difference found between these groups could be attributed only to the difference in wavelengths. Shrimp exposed to equal photophases, however, were not irradiated with the same amount of energy because the equipment to do so was not available. In any event, if a significant and similar response was observed in both groups irradiated with

UV-light and not in the white light controls, then the difference would be attributable to wave-length rather than photoperiod or energy.

Available light energy at the bottom of the aquaria was estimated by substituting the needed values into the equation expressing the extinction of radiation in sea water, *i.e.*, $I = I_0 e^{-kz}$ (Neumann and Pierson, 1966). The aquarium water depth (z) was 21 cm, the incident radiation (I_0) is given in the text, the absorption coefficient (k) was estimated by examining the spectral distributions of the lights used and converting the average percent transmission of the aquaria water for the wavelengths which contributed the most to absorbance ($A = \log T^{-1}$). The daily amount of energy received at the surface of the substrate in the aquaria was calculated to be approximately 11, 0.66, 22, and 0.74 joules cm^{-2} for Groups II, III, IV, and V, respectively. The energy received by Groups III (12:12LD-UV) and V (24:00LD-UV) is similar because the flashlight used during scotophase monitoring for Group III contributed almost as much energy as 12 hr of UV-light. Group I (00:24LD) received about 0.6 joules cm^{-2} daily and an additional 1.4 joules cm^{-2} once a week while measuring the shrimp's growth. The energy calculations for Group I are absolute maximums because the flashlight was on for no more than five minutes each day and not directed at any individual shrimp during that time.

The pH of the water was initially 8.2 and dropped to 7.2 for Groups I, IV, and V; and to 7.0 for Groups II and III. This is not considered to have a deleterious effect, as it is in the range of estuarine environments inhabited by the species at this age.

Results

Burying

The weekly cumulative number of shrimp counted above the substrate in each aquarium for each observation time is presented in Table I, and Table II presents the corresponding weekly cumulative number of shrimp in the aquaria. An

Table I

Weekly cumulative number of shrimp above substrate.

Group	I				II				III				IV				V			
Tank	1		2		3		4		5		6		7		8		9		10	
Time**	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s
1st week	46	46	40	46	02	61	04	63	01	57	01	49	19	28	18	34	08	08	07	06
2nd week	48	46	31	45	09	49	12	68	00	56	09	62	12	28	25	31	15	06	26	10
3rd week	50	50	48	59	03	55	01	58	01	57	03	66	05	15	09	23	14	26	16	21
4th week	47	53	54	45	05	59	02	45	00	61	00	62	14	19	26	26	28	21	17	22
5th week	43	52	46	54	06	45	02	36	03	60	00	54	21	26	20	17	05	12	05	14
6th week	44	45	42	42	03	43	02	32	01	45	01	42	15	09	15	20	05	12	09	12
7th week	33	44	39	37	07	39	01	30	03	29	04	32	11	13	21	23	12	07	12	11
8th week	29	29	25	27	04	40	03	29	01	18	04	33	07	06	12	26	09	15	10	19

** p = 1200–2400, s = 0000–1200.

Table II

*Weekly cumulative number of shrimp.**

Group	I				II				III				IV				V			
Tank	1		2		3		4		5		6		7		8		9		10	
Time**	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s
1st week	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
2nd week	70	70	70	70	66	67	70	70	66	67	70	70	70	70	70	70	70	70	70	70
3rd week	70	70	70	70	63	63	62	64	63	63	70	70	70	70	63	63	70	70	65	65
4th week	70	70	70	70	63	63	56	56	63	63	66	67	70	70	63	63	65	66	63	63
5th week	68	69	69	69	59	60	53	54	63	63	58	59	64	64	61	61	63	63	60	60
6th week	61	62	63	63	56	56	44	44	59	60	56	56	60	61	55	56	54	57	51	52
7th week	54	55	56	58	56	56	40	41	39	39	50	51	37	38	46	47	42	42	41	42
8th week	43	43	33	37	46	47	32	33	24	24	42	43	28	28	38	38	31	32	33	33

* The average number of shrimp per day in any tank for any given week may be obtained by summing the cumulative number of shrimp observed during the two observation times for that tank and week and dividing by 14.

** p = 1200–2400, s = 0000–1200.

ANOVA of the number of active shrimp per total shrimp is given in Table III. Significant differences were found to occur among the treatments, periods, times, period × time, treatment × period, treatment × time, and the treatment × period × time interactions. The mean number of shrimp above the substrate for the sources of variation are presented in Table IV.

An average of 69% of the shrimp of Group I (00:24LD) were visible above the substrate. This was significantly greater than the 24 to 44% average observed to be active in Groups II–V. Shrimp in Groups II and III were significantly more active (43%) than those of Groups IV and V (28%). The burying behavior of shrimp irradiated with a 12-hr photophase (Groups II and III) did not differ significantly, nor was there a significant difference between that of shrimp irradiated with 24-hr light (Groups IV and V).

The burying behavior of *P. duorarum* during the first and second two-week periods was not significantly different from that of the third and fourth two-week periods. Significantly more activity, however, was observed during period 2 (45%) than period 1 (41%), and highly significantly more activity was observed during period 4 (45%) than period 3 (40%).

Significantly more burying occurred from 1200–2400, *i.e.*, during the time coinciding with the photophase of Groups II and III. The average number of shrimp, for all groups, observed above the substrate from 1200–2400 and 0000–1200 was 25 and 59%, respectively.

Because mean shrimp activity was similar during the periods 1 and 3, and 2 and 4 (see period section of Table IV), the treatment × period interactions were analyzed individually for lunar influence rather than for the effects of the passage of time. That is, significance was tested for differences between periods within each treatment (see Table III). The orthogonal comparisons consisted of three tests for each treatment: periods 1, 3 *vs.* 2, 4; period 1 *vs.* 3; and period 2

TABLE III

ANOVA for cumulative number of shrimp above substrate (Table I) per cumulative number of shrimp (Table II).

Source of variation	df	Mean square
Treatment	04	0.45686**
T_1 *vs.* T_2–T_5	01	1.36949**
T_2, T_3 *vs.* T_4, T_5	01	0.39843**
T_2 *vs.* T_3	01	0.00090
T_4 *vs.* T_5	01	0.05863
Error a[1]	05	0.01073
Period	03	0.01366**
P_1, P_2 *vs.* P_3, P_4	01	0.00060
P_1 *vs.* P_2	01	0.01704*
P_3 *vs.* P_4	01	0.02333**
Time	01	2.32917**
Period × time	03	0.00629*
Treatment × period	12	0.01261**
T_1:P_1, P_3 *vs.* P_2, P_4	01	0.00914
T_1:P_1 *vs.* P_3	01	0.01307*
T_1:P_2 *vs.* P_4	01	0.00190
T_2:P_1, P_3 *vs.* P_2, P_4	01	0.00034
T_2:P_1 *vs.* P_3	01	0.01636**
T_2:P_2 *vs.* P_4	01	0.00193
T_3:P_1, P_3 *vs.* P_2, P_4	01	0.00024
T_3:P_1 *vs.* P_3	01	0.00014
T_3:P_2 *vs.* P_4	01	0.01223*
T_4:P_1, P_3 *vs.* P_2, P_4	01	0.00000
T_4:P_1 *vs.* P_3	01	0.00523
T_4:P_2 *vs.* P_4	01	0.02988**
T_5:P_1, P_3 *vs.* P_2, P_4	01	0.10155**
T_5:P_1 *vs.* P_3	01	0.00011
T_5:P_2 *vs.* P_4	01	0.00013
Treatment × time	04	0.60607**
T_1(00:24LD)	01	0.00868
T_2(12:12 LD-W)	01	2.21377**
T_3(12:12 LD-UV)	01	2.49134**
T_4(24:00 LD-W)	01	0.03543*
T_5(24:00 LD-UV)	01	0.00421
Treatment × period × time	12	0.00639**
Error b	35	0.002098
Total	79	

[1] Aquaria per treatment.
* Significant at $\alpha = 0.05$.
** Significant at $\alpha = 0.01$.

vs. 4. In Treatment I, significantly less activity occurred during period 1 (62%) than period 3 (70%); in Treatment II, highly significantly more activity occurred in period 1 (48%) than 3 (39%); in Treatment III, significantly more activity occurred in period 2 (48%) than 4(40%); in Treatment IV, highly significantly less activity occurred in period 2 (26%) than 4 (38%); and in Treatment V, highly significantly less activity occurred during periods 1, 3 (15%) than 2, 4 (31%). Difference for all other comparisons were not found to be significant.

TABLE IV

Mean shrimp above substrate.

Source of variation		Observations (n)	Mean shrimp above substrate
Treatment	I (00:24LD)	16	0.69
	II (12:12LD-W)	16	0.44
	III (12:12LD-UV)	16	0.43
	IV (24:00LD-W)	16	0.32
	V (24:00LD-UV)	16	0.24
Period	1 (26 Aug–09 Sept)	20	0.41
	2 (10 Sept–23 Sept)	20	0.45
	3 (24 Sept–07 Oct)	20	0.40
	4 (08 Oct–21 Oct)	20	0.45
Time	1200–2400	40	0.25
	0000–1200	40	0.59

Treatment × period*	I	II	III	IV	V
26 Aug–09 Sept	0.62	0.48	0.42	0.35	0.15
10 Sept–23 Sept	0.73	0.46	0.48	0.26	0.31
24 Sept–07 Oct	0.70	0.39	0.43	0.30	0.16
08 Oct–21 Oct	0.69	0.43	0.40	0.38	0.32
Treatment × time**					
1200–2400	0.66	0.07	0.04	0.28	0.22
0000–1200	0.71	0.81	0.83	0.37	0.25

* Number of observations (n) = 4.
** Number of observations (n) = 8.

The activity of the treatment × time interaction was found to be significantly different. Activity was highly significantly greater from 0000–1200 for Groups II and III, and significantly greater for Group IV. The mean number of shrimp visible per number of shrimp present during 1200–2400 and 0000–1200 was 0.07 and 0.81, 0.04 and 0.83, and 0.28 and 0.37 for Groups II, III, and IV, respectively. No significant differences were found between the times for Groups I and V (Table IV).

Orthogonal comparisons for the significant differences found for the period × time and the treatment × period × time interactions were not computed, because the time factor is primarily responsible for their differences.

Molting

The weekly cumulative number of exuviae collected in each aquaria for each observation time is presented in Table V. Table VI is an ANOVA of the cumulative number of exuviae per cumulative number of shrimp (Table II). Tables VII, VIII, and IX give the mean molt rate for the periods, the times, and the treatments × time interactions, respectively.

Highly significant differences were found among the periods. The molt rate was significantly greater during the second period (4.2%) than the first (2.5%) and during the latter two periods (4.0%) compared to the first two periods (3.3%).

TABLE V

*Weekly cumulative number of exuviae.**

Group	I				II				III				IV				V			
Tank	1		2		3		4		5		6		7		8		9		10	
Time**	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s	p	s
1st week	0	2	0	1	4	0	6	0	2	0	1	0	0	0	0	0	6	0	3	0
2nd week	0	4	0	3	5	1	3	0	5	0	4	0	2	0	5	2	4	0	6	1
3rd week	4	1	6	1	5	0	6	0	8	0	5	0	4	3	1	6	3	3	5	3
4th week	2	1	1	0	6	0	4	0	5	0	4	0	4	4	3	2	4	1	2	2
5th week	3	1	7	1	6	0	4	0	5	0	6	0	1	3	1	3	1	5	3	3
6th week	5	1	2	1	4	0	4	0	4	0	3	0	3	2	1	5	0	4	1	2
7th week	2	0	5	1	7	0	3	0	3	0	4	0	1	1	1	2	2	2	2	2
8th week	4	0	4	1	2	0	3	0	2	0	3	0	2	1	0	3	1	0	0	1

* Exuviae found during the photophase probably resulted from ecdysis during the scotophase and vice versa.

** p = 1200–2400; s = 0000–1200.

No significant differences were found between the third and fourth period molt rates (Table VII).

Highly significant differences were found between times. From 1200–2400, the mean number of exuviae found per shrimp for 40 observations was 0.055; and that from 0000–1200 was 0.018.

TABLE VI

ANOVA for cumulative number of exuviae (Table V) per cumulative number of shrimp (Table II).

Source of variation	df	Mean square
Treatment	4	0.000139
Error a[1]	5	0.000085
Period	3	0.001191**
P_1, P_2 *vs.* P_3, P_4	1	0.000855*
P_1 *vs.* P_2	1	0.002714**
P_3 *vs.* P_4	1	0.000003
Time	1	0.026514**
Period × time	3	0.000317
Treatment × period	12	0.000189
Treatment × time	4	0.005824**
Group I	1	0.003648**
Group II	1	0.025297**
Group III	1	0.019937**
Group IV	1	0.000389
Group V	1	0.000537
Treatment × period × time	12	0.001123**
Error b	35	0.000143
Total	79	

[1] Aquaria per treatment.

* Significant at $\alpha = 0.05$.

** Significant at $\alpha = 0.01$.

TABLE VII

Mean death and molt rate per period.

Period	Observations	Mean death rate	Mean molt rate
1	20	0.001	0.025
2	20	0.002	0.042
3	20	0.008	0.040
4	20	0.018	0.040

The treatment × time interactions were found to be highly significantly different. The mean percent of exuviae found per shrimp for the times 1200–2400 and 0000–1200 was 4.8 and 1.8, 8.0 and 0.1, and 7.1 and 0.0 for Groups I, II, and III, respectively. No significant differences in the molt rate were found between the times 1200–2400 and 0000–1200 for Groups IV and V (Table VIII).

Orthogonal comparisons were not computed for the highly significant differences found among the treatment × period × time interactions because this difference was caused mainly by the difference in the treatment × time interaction.

DISCUSSION

The shrimp's activity above the substrate was inversely related to the amount of exposure to light. This is substantiated by the fact that shrimp were significantly more active in the absence of light (Group I) than those in the remaining groups, which were subjected to some light exposure, and significantly more active in groups irradiated with 12-hours of light (Groups II and III) than those exposed to constant light (Groups IV and V). The activity of the groups of shrimp exposed to equal durations of light, but different wavelengths, was similar (refer to treatment section of Tables III and IV). On the whole, most activity occurred from 0000–1200, *i.e.*, during the scotophase of Groups II (12:12LD-W) and III (12:12LD-UV).

TABLE VIII

Mean molt rate for treatment × time interaction.

Treatment	Time	Observations	Mean molt rate
Group I	1200–2400	8	0.048
Group I	0000–1200	8	0.018
Group II	photophase	8	0.080
Group II	scotophase	8	0.001
Group III	photophase	8	0.071
Group III	scotophase	8	0.000
Group IV	1200–2400	8	0.031
Group IV	0000–1200	8	0.041
Group V	1200–2400	8	0.044
Group V	0000–1200	8	0.033

TABLE IX

Number of exuviae per shrimp.

Group	I	II	III	IV	V
Total exuviae	64	73	64	66	72
Average number of shrimp per group for 56 days	18.06	16.25	16.64	16.73	16.46
Exuviae per shrimp for 56 days	3.54	4.49	3.85	3.94	4.37

More exact information about the time and treatment effects is obtained from the orthogonal comparisons of their interactions. The average burying behavior between 0000–1200 and 1200–2400 was not found to be significantly different for shrimp in Group I (00:24LD). That is, an endogenous rhythm of burying activity was not found to exist in shrimp kept in constant darkness. If additional data had been collected with 1.5 hours after sunrise or sunset, an endogenous rhythm may have been found, because evidence was obtained from Group I to support the existence of an endogenous molt rhythm with most molts being found from 1200–2400 (Tables V and VIII). Thus, the preclusion of light does not necessarily interrupt all rhythms. Wickham (1967) found a nocturnal circadian activity pattern for *P. duorarum* after 100 hours of continuous darkness, and Racek (1959) found that *P. plebejus* maintained a nocturnal activity pattern in constant darkness for a month. Dall (1958) found that *Metapenaeus mastersii* exhibited a nocturnal activity pattern in constant darkness for at least seven days. Neither Dall (1958) nor Racek (1959) gave information about the procedures for keeping the tanks dark or how the shrimp were observed. Fuss and Ogren (1966) kept *P. duorarum* in continuous darkness for three days and found the shrimp more active the entire time, but this activity declined with the passage of time. After several weeks to natural light acclimation, *P. semisulcatus* maintained distinct circadian burying activity for four days in constant darkness (Moller and Jones, 1975). They found the emergence of *P. semisulcatus* to be more closely synchronized with "expected" dusk than re-entry to "expected" dawn. No endogenous burying rhythm, however, was found for *P. monodon* acclimated and tested in a similar manner (Moller and Jones, 1975). Brown (1961) discusses other activity cycles of crustaceans held under the continuous absence of light.

The influence of light on the burying behavior is most evident in the treatment × time interaction of Groups II (12:12LD-W) and III (12:12LD-UV). Almost all activity exhibited by the shrimp occurred during the scotophase (0000–1200) with very little being observed during the photophase. Thus it appears that light is the most important factor governing activity of *P. duorarum* as reported by Fuss and Ogren (1966). Certainly the significant difference between the times 0000–1200 and 1200–2400 (see Tables III and IV) is due primarily to the activity differences found in Groups II and III. Hughes (1968) found that over 90% of the shrimp present emerged from the substrate within 45 minutes after the light-dark transition. He also found the light-dark transition to be the Zeitgeber for emergence, or at least a principal factor of the Zeitgeber. In addition, he found evidence of a 24-hour feeding rhythm. When food was made available

immediately after the light-dark transition, the burying rhythm could be resynchronized to an eight-hour advance of the light-dark transition within four days. When food was not made available after the transition, resynchronization took six days (Hughes, 1968). Moller and Jones (1975) also found light to be the main synchronizer for activity for 24-hour periods. They monitored the activity patterns of *P. monodon* and *P. semisulcatus* irradiated with 06:18LD, 12:12LD, and 18:06LD photoperiods of white light, and found that the periods of activity were closely related to the scotophase. Similar results were obtained for *P. duorarum* which were subjected to a natural photoperiod and a photoperiod with a 6 hr advance of the scotophase (Wickham and Minkler, 1975).

An examination of the daily activity data shows that in the present experiment, shrimp of Groups II and III resynchronized almost immediately; during the first week, an average of over 88% of Group-II shrimp and 75% of Group-III shrimp were observed above the substrate during each monitoring time of the scotophase, and less than 2 and 4%, respectively, were observed during the photophase. Therefore, the change of the timing of the light-dark transition from that of natural conditions is considered to affect only the timing of the burying or emergence.

A circadian burying rhythm was found for shrimp continuously irradiated with white light (Table III, treatment × time section). Shrimp of Group IV (24:00LD-W) were significantly more active from 0000–1200, thus coinciding with the activity patterns of Groups II (12:12LD-W) and III (12:12LD-UV). Although the light conditions were constant throughout the experiment, there was a semidiurnal feeding pattern to which the shrimp could have adjusted for their burying pattern. But if feeding became the predominant Zeitgeber in the presence of constant light, a similar burying pattern would be expected to occur also in Group V (24:00LD-UV). Because a circadian burying pattern was not found in Group V, it might be possible that particular wavelengths of light are needed for the continuance of the circadian burying behavior or that continuous UV-light might interfere with a burying rhythm. Fuss and Ogren (1966), Wickham (1967), and Wickham and Minkler (1975) found evidence of circadian burying that coincided to natural burying for *P. duorarum* irradiated with continuous light, and Hughes (1968) found a circadian periodicity of burying for *P. duorarum* under a constant low light intensity after three consecutive days. Thus white light might be necessary for maintaining a circadian burying pattern.

The burying activity differences between the two monitoring times for Group IV (24:00LD-W) are obvious only during the first three weeks (Table I). Circadian rhythms are not always 24-hour cycles (Brown, 1973), and it is possible that the burying activity was changing from 0000–1200 to 1200–2400 during the later four to five weeks of the experiment (Table I). Wickham (1967) found that under constant dim light for 72 hours, activity peaks for *P. duorarum* occurred approximately one hour later each night and noted that these peaks "progressed daily with the tides." In the absence of natural tidal and solar rhythms, the circadian burying activity could have progressively diminished so that by the fourth week, the activity patterns were no longer evident. More frequent observations per 24 hours would be necessary to verify this.

No significant differences in activity were found between the first two periods compared to the last two periods. There was, however, significantly more activity

in the second period compared to the first, and significantly more in period 4 compared to period 3. Thus, there appears to be little change in burying behavior for the overall experiment, but there are changes among the periods. An average of just over 40% of the shrimp was visible during both periods 1 and 3, and periods 2 and 4 both had an average of 45% visible. Periods 1, 2, 3, and 4 covered the intervals from August 26 to September 9; September 10–23; September 24 to October 7; and October 8–21, 1969, respectively. The full and last-quarter moon occurred during periods 1 and 3, and the new and first-quarter moon occurred during periods 2 and 4 (*American ephemeris and nautical almanac for the year 1969*). Overall, shrimp were most active during periods 2 and 4 (*i.e.,* during the new and first-quarter moon), but this difference was found to be present only in Group V (24:00LD-UV). Significant differences were mixed for the other interactions and are not easily explainable. Activity of Groups I (00:24LD) and II (12:12LD-W) differed between periods 1 and 3; shrimp were more active in Group I during period 3 and more active in Group II during period 1. Groups III (12:12LD-UV) and IV (24:00LD-W) exhibited activity differences between periods 2 and 4. Again opposite reactions occurred: most activity occurred in period 2 for Group III and in period 4 for Group IV. There seems to be no correlation for moonphase, photoperiod, energy levels, or wavelengths except for Group V.

In field studies, Fuss (1964) and Fuss and Ogren (1966) found that activity was less during periods of increased moonlight, though aquarium studies under continuous dark or light during the full and last-quarter moon phase revealed no strong evidence of lunar rhythmicity. Wickham (1967) found no apparent differences of burying behavior under constant conditions during the new and full moon, and Racek (1959) testing for lunar effects on the activity of three species of prawns in aquaria exposed to a natural photoperiod and continuous darkness for a month failed to find any differences. Previous studies of penaeid shrimp behavior and moon phase correlations (Beardsley and Iversen, 1966; Fuss, 1964; Fuss and Ogren, 1966; Racek, 1959; Wheeler, 1937; and Wickham, 1967) seem to support the conclusion that lunar phase exhibits an influence *in situ*, but that this influence is lost in aquaria studies. An exception is found in Aaron and Wisby's (1964) work in which a correlation was observed between moon phase and photoactivation of *P. duorarum* with maximum and minimum photoactivation being observed during the full and new moon, respectively. Apparently this is the only other reported evidence of possible lunar influence on behavior of *P. duorarum* in aquaria. The relationship between moon phase and shrimp activity for Group V indicates that there may be a lunar effect, but more controlled experiments are needed before any conclusions can be made.

The molt data obtained from this experiment did not reproduce that obtained in the preliminary study. The preliminary experiment, however, was only a cursory investigation, and it is believed that the molt data reported here are a truer indication of the effects of the selected photoperiods and wavelengths on the molting of shrimp.

Because Bliss and Boyer (1964) showed that light affects molting through the eye in at least one decapod crustacean (*Gecarcinus*) and because the X-organ, sinus-gland complex is associated with the eye, it is assumed that the

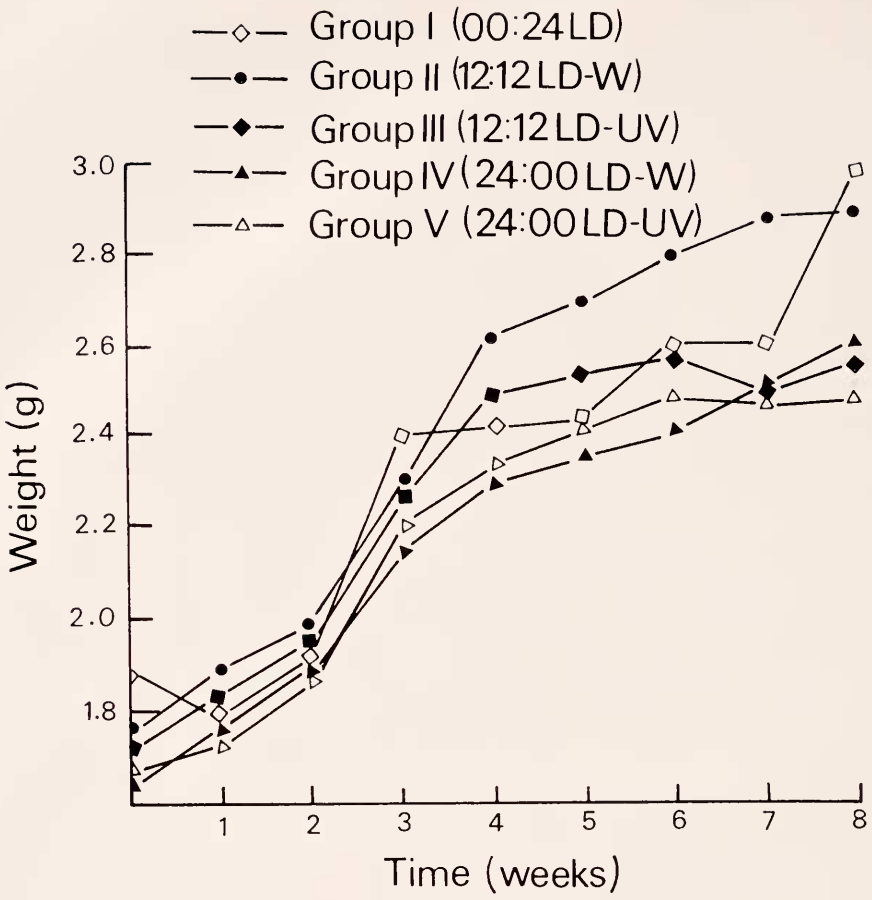

FIGURE 1. Growth (average weight).

MIH may be photoregulated (Aiken, 1969). Photoregulation of the X-organ may be achieved in one of four ways: by a particular wavelength, the amount of energy, the photoperiod, or through combined interactions.

The equivalent number of total exuviae among the groups indicated that the photoperiod, wavelength, or a combination did not markedly affect the rate of molting, but rather, influenced the time molting took place. Most exuviae were found from 1200–2400, but because monitoring of all the aquaria occurred not more than two hours after the scotophase-photophase change of Groups II and III, it is assumed that most molts took place from 0000–1200.

The mean molt rate is similar throughout the experiment (Table VII) except for period 1 during which the molt rate was lower than that in period 2. Presumably the lower molt rate was due to acclimation to laboratory conditions. Periods 1, 3, and 4 showed low growth rates (*i.e.*, 0.16 g *vs.* 0.52 g for period 2), but only period 1 showed low molt rates (Table VII). Therefore, either shrimp

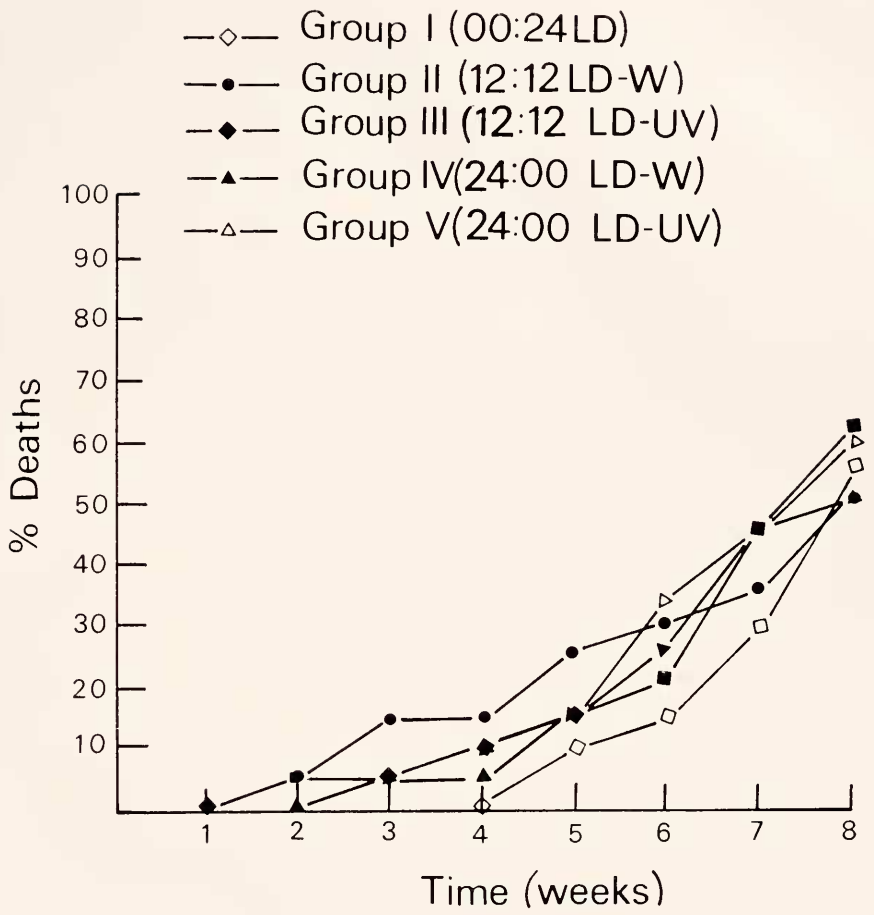

FIGURE 2. Cumulative per cent deaths.

were molting without growth, as reported by Eldred, *et al.* (1961) or the faster growing individuals were dying. The latter possibility is supported because mortality increased markedly during the period that the growth rate decreased (Figs. 1 and 2, Table VII). The possibility that the faster growing shrimp died during ecdysis is also supported by the results of Aiken's (1969) experiments, in which the freshwater crayfish, *Orconectes virilis*, died during ecdysis induced by alteration of the photoperiod. Similar effects may have occurred in the present experiment. On the other hand, the molt rate did not decline during this period, suggesting that the molt processes continued throughout the experiment (Fig. 3).

The high number of exuviae being found between 1200–2400 in Group I (00:24LD), indicates that *P. duorarum* may have an endogenous molt rhythm in the absence of light. Similar molt differences for the monitoring periods were not found in Groups IV (24:00LD-W) or V (24:00LD-UV), so continuous light

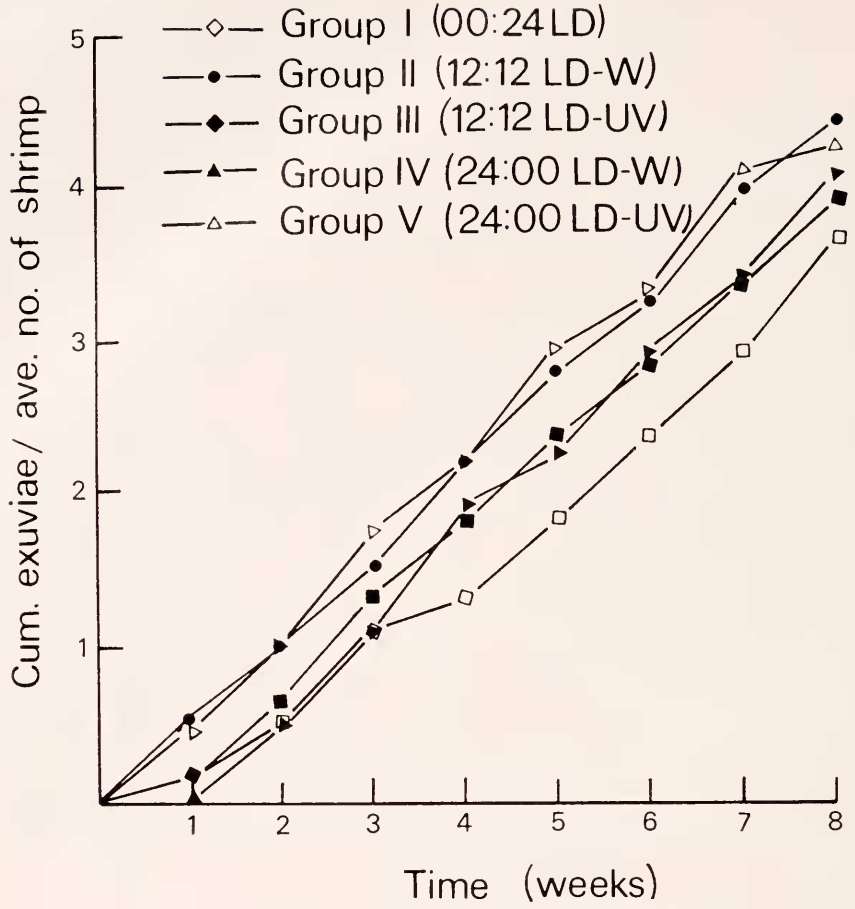

FIGURE 3. Cumulative exuviae per average number shrimp.

may interfere with this rhythm. A cursory examination of the daily exuviae data show no trends that would be negated by the two-week summation of the data. It cannot be determined from the present data whether the molt rhythm obtained for Group I is a fluke, but it should be pointed out that nearly twice as many exuviae were found from 1200–2400 than 0000–1200 in both tanks of Group I (see Table V). Dall (1965) reports that the molting of *Metapenaeus* sp. is inhibited in constant dark or constant light.

Groups II and III showed a highly significant difference of ecdysal activity between the two monitoring times. All exuviae except one were found during the photophase, 1200–2400 (Table V). Eldred (1958) and Dall (1965) found all molts to take place late at night or early in the morning. This nocturnal molting behavior probably serves a protective function as well as coinciding with the shrimp's activity. Shedding an exoskeleton is an extremely vivacious process

(Eldred, 1958) and would be difficult if not impossible to do while buried during the day. Newly molted shrimp are in their most vulnerable state and therefore darkness might offer some protection from predators that hunt by daylight.

Because there was no significant difference among treatments, an average molt rate was obtained by dividing the total number of exuviae by the average total number of shrimp (Table IX). This resulted in an average molt rate of one exuvium per 14 days for 55–73 mm shrimp (total length), approximately the rate mentioned in past studies. Known intermolt periods for penaeid shrimp range from 12–14 days (Dall, 1965; Eldred, *et al.*, 1961; Hudinaga, 1942), and for caridean shrimp from 13–36 days (Jefferies, 1964; Lloyd and Yonge, 1947; and Reeve, 1969). Nouvel-van Rysselberge (1937) found that the growth rate is less in aquaria conditions than in nature, thus a fortnightly exuviation in aquaria is probably less than that in natural conditions.

Because *P. duorarum* probably exuviates seasonally in its northern range, it may have MIH (see Carlisle, 1954). Therefore, one would expect the different photoperiods used to affect the molt rates. The average molt rates for the 56 days, however, were not significantly different (Tables VI and IX) and cumulative exuviae per average number of shrimp in different groups differ only slightly (Fig 3). Thus, the different lighting conditions affected the time of molting and apparently had little or no affect on increasing or decreasing the molt rate.

Generally, groups of shrimp exposed to an equal duration of light responded similarly, indicating that the shrimp were responding to a wavelength of light common to both bulbs used or that the length of the photophase, regardless of the wavelength, caused the response. Both the white light and the UV-light contain 320–400 nm wavelengths, and it may be this portion of the spectrum to which the shrimp were responding. Except for the endogenous activity differences found in Groups IV and V, the burying behavior as well as the molt rate were similar between groups with an equal photoperiod timing, *i.e.*, Groups II and III and Groups IV and V. It is believed, therefore, that the shrimp were responding to the length of the photophase (or scotophase) and not to the wavelength of light.

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Summary

1. Shrimp activity was inversely related to the amount of light exposure.

2. Shrimp responded to a 12-hour photophase of white light and UV-light in a similar manner. The light-dark transition is apparently the Zeitgeber, and the burying rhythm can be readily changed with a change in the light-dark transition.

3. A circadian burying rhythm was found for shrimp kept in continuous white light; no rhythm was found for shrimp kept in continuous darkness or UV-light.

4. Evidence was obtained to support a lunar influence, with shrimp of Group V being more active during the new and first-quarter moon.

5. Burying activity between the first and last four weeks of the experiment was similar.

6. No significant differences among the groups' overall growth rates or molt rates were found for the four two-week periods.

7. The photoperiod influenced the time rather than the rate of molting. Groups II and III molted highly significantly more during the scotophase. Group I with no light molted highly significantly more during the time interval corresponding with the scotophase of Groups II and III, indicating presence of an endogenous molt rhythm.

8. Molt rate did not decline in the latter half of the experiment as did the growth rate.

9. Deaths approached 50% for all groups at the end of eight weeks.

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